All shelves in the culture room and greenhouse are washed between crops with Physan @ 9 75 ml/3 liters of water and periodically the greenhouse side-walls are also washed.

Running this profitable tissue culture lab has been a very worthwhile endeavor, climaxing 40 years of growing. I regularly review over 40 technical journals at Cal-Poly in Pomona, copying those articles on tissue culture and card indexing same. We feel that our achievements in tissue culture is due to our life-long interest and hobby in plants. One of our customers in Anaheim is starting his own lab under our training.

OVERVIEW OF TISSUE CULTURE AT K. M. NURSERY

JIRO MATSUYAMA

K.M. Nursery

Carpinteria, California 93013

We, at K M. Nursery, have been involved in tissue culture since 1969. We have met and talked with many researchers and commercial producers from almost every country in the world about the problems we have encountered.

The foreign countries are working on mostly vegetative crops, such as those grown for paper pulp and number, especially in the smaller countries, while in the United States it seems we are producing mostly ornamentals commercially, although much research is going on in tissue culture of herbaceous crops So it will not be very long before many of the important herbaceous plants will be produced through tissue culture

Many nurserymen do not understand propagation by tissue culture although many articles have been written and talks have been given by speakers on this subject. Many people think tissue culture is as simple as mixing some media formula for all cultures, then placing shoot tips in a test tube and culturing it in an ideal room temperature and in a few weeks having sizable multiple plantlets. This is wishful thinking. It takes many man-hours of research for each species and cultivars that you are going to culture for commercial production, especially for hardwood plants.

When first starting commercial production sanitary conditions aren't a problem as laboratory equipment and the culture room are new and easily kept clean. But as time goes on, in mass propagation contamination will appear.

I have always said research is one thing that we all need.

Commercial production is something else when tens of thousands of cultures are involved every day.

In January, 1975, we were able to get started in commercial tissue culture production in our own laboratory, with the help of Dr. Toshio Murashige of the University of California, Riverside, who was instrumental in getting us started. Without his help, advice, and encouragement we couldn't have gotten started or come as far as we have.

All tissue cultured plants produced in our laboratory are transplanted into 1-gallon, 5-gallon, or 15-gallon containers to be sold as outdoor landscape material. Any excess we produce is sold as 2¼" pot liners.

In over five years of commercial production, we are constantly doing research to see if we can get better multiplication, a superior rooting system on woody plants, with quicker and better ways of hardening off.

Most laboratories are producing indoor house plants. With these there are not many problems compared to woody landscape plants. First of all, in woody plant you have contamination from the soil This is natural because they are grown directly in or on the soil, while house plants are grown indoors and isolated from the ground, so there is less chance of contamination.

Woody plants are more inconsistent in the multiplication stage than soft wood materials. Many times contamination is within the tissue itself, which makes it harder to clean during culturing. There are times when the disinfection process has to be repeated 3 to 5 times to get the material clean. The best procedure is to isolate the mother stock plants off the ground — kept indoors if possible. Keep it under drought condition and irrigate only when needed and from the bottom only. By doing this for 3 to 4 months there is a better chance of getting a clean culture. Contamination during culture has to be watched closely. It is easier to prevent contamination than to control it after infection. This is not only viruses and bacteria but includes thrips, mites, web gnats, etc. Some of these insects are so small and transparent that it is almost impossible to detect them without a 25× power microscope.

Next, rooting is a problem on hard-woods. But it is just a matter of time before it will be solved.

I think the major problem, at the present time, is the hardening off after rooting in the liner stage, before it goes out to the outside environment. Humidity, temperature, light, mist control, rooting, fungicides and fertilizers, have a lot to do with survival of the plants.

Media — originally, we were making our media from

scratch. Later, we started to buy pre-mix media to save time and labor. For a while it seemed like a good pre-mix, but later we started to see some difference in the growth of some cultures. So we did some tests and found that the company had forgotten to include some chemical in the pre-mix or they had substituted some chemical with inferior products After our tests we decided to go back to making our own media.

We have cultured many plants species. But, for reasons of costs, in time and labor, we have found that the conventional method is the better way for propagation of some species of plants.

Commercial production of the following species has been accomplished:

Agapanthus 'Mood indigo'
Anthurium
Ophiopogon planiscapus 'Arabicus'
Black mondo grass
Ophiopogon japonicus, Dwarf mondo grass
Clerodendron thomsoniae
FERNS
Adiantum raddianum (Syn *A cuneatum*)
Alsophila australis
Aspidium capense (*Rumohra adiantiformis* ? Bot ed)
Davallia trichomanoides
Dicksonia antarctica
Nephrolepis exaltata
Woodwardia fimbriata
Ficus benjamina
F decora
F decora 'Burgundy'
F pandurata
Hemerocallis 'Aztec Gold'
Howea forsterana
Nandina 'Royal Princess'
Nandina 'Compacta Nana'
Nerine
Photinia × *fraseri*
Sequoia sempervirens
S sempervirens 'Santa Cruz'
Simmondansia chinensis (Syn , *S. california*)
Tupidanthus calyptratus
Zanthoxylum piperitum

MODERATOR BRUCE BRIGGS: We have time for a few questions. The first is for Dr. Cheng.

VOICE: How do you obtain your explants for your tissue cultures?

TSAI YING CHENG: Tree species are very different from herbaceous plants. What we do is to provide enough chilling treatment and then bring the stock plants into the greenhouse and force them to break dormancy. Then we force the new shoots to grow very rapidly and then we take these shoots for tissue culture use. Now, after we remove the shoots from rapidly growing trees, we remove the leaves and cut the stems into appropriate sizes and then sterilize with Clorox. Then we put them into a conditioning medium because most of the stem pieces we obtain from trees, even after Clorox treatment, are often still contaminated with microorganisms. So, by having this conditioning step, we can eliminate all the contaminated ones, and just choose clean materials for shoot multiplication. Also

under these conditions we try to treat plant materials in such a way that they are homogeneous so that when we put them into the shoot multiplication stage they give us a more homogeneous type of response. In addition, when you have plant materials under tissue culture conditions, often you don't have to put them in shoot multiplication medium right away. You can maintain them in a conditioning medium and hold them for a time. It is a convenient step for us. Therefore, in the preconditioning treatment we use a basal medium which contains very small amounts of auxin and cytokinin. I am talking in terms of something like ⅒ of 100 ppm of IBA and BAP. Or we put them in the basal medium without any hormones and later transfer them into a medium containing hormones, depending on the plant species or cultivars. We have to decide which is the best method to use. The conditions for the preconditioning treatment are 16 hours photoperiod, 200 foot candles, and 20°C temperature.

WES HUMPHREY: Jiro Matsuyama, you showed a slide of *Howea forsterana*. You are doing some work on that in tissue culture with some success?

JIRO MATSUYAMA: The reason I am working on *Howea* is not to get multiplication; it is to cut down on seed germination time. It is embryo culture. I can save 50% time on germination. You have to know the correct time to excise the embryo. That is the trick to it. You don't wait for the seed to get ripe. The older the seed the harder it is. Doing embryo culture in the culture tube is one thing, but getting it out into the light is another story. It is real hard. We are working on it right now. We have quite a bit of it going, as far as that goes, in the culture tube. Getting the seedlings into the outside is the problem. I think that is what most of the production people are having a problem with.

LES CLAY: Dr. Cheng, have you had any experience in working with the Japanese maple?

TSAI YING CHENG: Well, grafting and budding — yes. You are asking a question that I am not supposed to answer. Since you ask the question, I work for the Oregon Graduate Center and my personal interest and the Center's interest has to be differentiated very clearly. Japanese maple has been my interest for years. Since I am doing tissue culture at the Center so what I can do with Japense maple is to try to improve the conventional methods. I am doing cuttings, grafting, budding with Japanese maple with quite successful results. But tissue culture, I have to talk to the Center to get approval before I can do it.

VOICE: What type of gibberellic acid was used in your work?

TSAI YING CHENG: I use GA_3 at 10 ppm. With maples we have to make sure that the cytokinin is on the lower side. The

high side is inhibitory, the proper balance of the three hormones (auxin, GA, and cytokinin) is very critical.

BRUCE BRIGGS· You say that cytokinins for Japanese maple should be on the low side?

TSAI YING CHENG: I would say about 0.1 or 0.2 ppm. The maple is a very striking example of the GA effect. It is so clear cut. You can see it in one week, with or without GA_3. It is very clear cut.

BRUCE BRIGGS: The problem you get into with GA is that you stimulate a plant but then you find it hard to put roots on the other end similar to what we have in cuttings. We sometimes had to quit using GA on plants because the cuttings didn't root. I am sure through tissue culture we will solve this problem — either through light or temperature or through a rest period or someway where we can destroy the GA and get some roots to develop

VOICE: Jiro, are you having good results on all of your redwoods, or is there a difference among cultivars?

JIRO MATSUYAMA: Well, mostly it is still in the research stage. I don't call it actual production yet. It doesn't come out the same all the time; we raise quite a few, but still I call it research. I am working with *Sequoia sempervirens* 'Santa Cruz'. So far it seems like it is pretty successful, But I can't say yet. A lot of these plants may be worth propagating through tissue culture. If it isn't we just quit. We do it through the conventional method of propagation; it is a lot easier and cheaper

RALPH SHUGERT: Tsai, I have two questions on Norway maples. One, does it make any difference what the explant is? Are you taking stem or leaf, whatever? Number two, after you get the rooted shoots, what about the stage until the nurseryman plants it out in the field as a one year liner? How are you handling that?

TSAI YING CHENG: The second question, I cannot answer very well because I am not involved in the commercial end of it. Maybe Bob Ticknor can answer you second question. The first question; we prefer to use stem explants. Even using stems we got lots of contamination. In using leaves, they are more tender when you go through Clorox treatment so you get lots of bruises So we prefer to use stems and, if necessary, at the conditioning stage we force the stems to produce other shoots, so will have new stems and leaves from there.

RALPH SHUGERT: So you are taking one year wood, really?

TSAI YING CHENG: Yes, the stem will be new growth.

RALPH SHUGERT: Does juvenility enter into this?

TSAI YING CHENG: Because all the trees are mature, I am not sure that they return to juvenility.

RALPH SHUGERT: OK, now how about the handling of the stock plants?

TSAI YING CHENG: Well, I got my maples from McGill Nursery. They gave me small size trees — just big enough to put in the greenhouse.

RALPH SHUGERT: Would it be a one-year whip, a one-year bud?

TSAI YING CHENG: Yes, about one year. I don't want to handle big trees. Small ones are much easier for me. Sometimes I cut the terminal buds off, and strip all the leaves to force the lateral shoots to grow. I take new growth from these.

BRUCE BRIGGS: Martin Crehan, you have been doing quite a lot of literature research on tissue culture propagation and keeping data on new plants and new material. Are you keeping up with the literature right now? You might like to comment on what you are doing in this area.

MARTIN CREHAN: I am located close to the California Polytechnic University at Pomona; when the idea first came to me, I did all of my studying at Cal Poly LIbrary. It is just a hobby with me. I copy most articles that are found in about forty different publications that are available at the Cal Poly Library. I xerox them, bring them home, and index them; I have a buddy up at Carpenteria, California, and I usually send them up there to him. He xeroxes what he needs in his lab and sends them back. We have numerous visitors and, believe it or not, they are from all over the world, from Taiwan, Australia, and New Zealand. When the American Society of Horticultural Science Conference was at Fort Collins, Colorado, we had three Ph.D's stop in during one day Most of the students at Cal Poly that want to do senior projects in tissue culture will come over and copy a lot of the reference work. It is really just a hobby with me, but it is a seven year collection, so far. I am starting to run out of file cabinets.

BRUCE BRIGGS: If we were to write you about some particular work that you might have information on, would you answer?

MARTIN CREHAN: Yes.

BRUCE BRIGGS: So, you members that have some new plant and are looking for some new data, here is a source of information.

Now, I would like to have all three of you comment on this problem. Do you feel we may have a problem in genetic breakdown in tissue culture? If we do have such a problem, how are

we going to solve it? I am talking about genetic variation — crooked leaves, stems, and such things that people don't like to see appearing in asexual propagaiton. We want to have a plant that comes true to name.

TSAI YING CHENG: Yes, I am very concerned about it. So what I suggest, perhaps, is to use hormones at the lower concentrations, because we know that to use high hormone concentrations you tend to induce a change in the chromosome numbers. Second, try to bring in new materials as often as you can so make sure you are not continually propagating a mutant.

MARTIN CREHAN: Bruce is talking about mutations. When we first started working with tissue culture, we ended up with the most beautiful mutations you have every seen. I have a bank in back of the greenhouse I built up with those mutations. When the problem was referred back to Dr. Murashige then, it was found out that in the second stage, the multiplication stage of the ferns, we were causing mutations. The recommendations then were that we could repropagate in tissue culture five, six, or seven times, but research at the University showed that the problem was alleviated by repropagating during the second stage only three times.

BRUCE BRIGGS: I think that Dr. Cheng did some work a while back — it is reported in the *Proceedings* — where she showed the chromosome counts didn't vary too much between tissue culture and normal propagaiton, which might be some indication. Have you changed you mind on that, Tsai, or have you checked anymore chromosomes?

TSAI YING CHENG: I haven't checked, but we have transplanted many tissue culture plants into the greenhouse and morphologically they look very good.

BRUCE BRIGGS: Jiro, on the same subject then, can we go the other way? If you are studying a new plant, you may have better results if you use cytokinins at a very high level so you can get shoot development, which means you get more shoots. But in the process of doing it there is the possibility of more mutations. Now, do you feel that we would be better to start off that plant slow, or would it be better off to go high in the cytokinins to get more shoots and then take it down to a low level. Or is there any difference?

JIRO MATSUYAMA: Well, when you are first starting out on tissue culture, you can use about six different kinds of media. Variations can occur; watch and see what happens. We try to get the minimum callus possible, but get roots to form. That is the main thing. If you get too much callus, you get too much root but then you know there is no top to it. The main thing is to have

shoots and roots. Shoot multiplication and rooting too. You have to have both.

VOICE: Has anyone produced fruit trees successfully by tissue culture methods?

TSAI YING CHENG: Well, the fruit trees I worked with was on understocks, not fruiting cultivars.

BRUCE BRIGGS: Dr. Anderson at Mount Vernon, Washington, has 'Red Delicious' in tissue culture; he is working on a project with several of the spur-type sports. At the present time, I am not sure that he has any tissue-culture grown trees back to the grower but he does have them growing. He has a rooting problem with the shoots, but he is working on that. They do not respond as well as the rootstock types. But I know that they are going to solve this problem.

ARDA BERRYHILL: Jiro, you mentioned that some things you grow are more suitable by conventional propagation than by tissue culture. Out of curiousity, could you mention those that you find more suitable for conventional propagation than by tissue culture?

JIRO MATSUYAMA: Well, like I said before, *Photinia* × *fraseri*, is one You can grow that by tissue culture like a weed, but it is not worth it. If you propagate by conventional methods, it is just as easy. You can get all you want.

ARDA BERRYHILL: It is a matter of economics.

JIRO MATSUYAMA: Yes, that's right.

VOICE: Jiro, could you elaborate on what you said on variations in the sequoia? What were the variations that you saw?

JIRO MATSUYAMA: What I meant was that you find a mother stock tree with a lot of close nodes, good tree shape, and then you try to tissue culture from that strain.

RALPH SHUGERT: Bruce, has anybody tissue-cultured *Taxus*?

VOICE: I think in France they are working with *Taxus*, yellow pines, and cedars at the Phytotron just outside Paris.

RICHARD SMITH: Dr. Cheng, you mentioned the virus load in certain woody ornamentals you are working with. Have you made any attempt to free these plants of virus?

TSAI YING CHENG: Yes, but I haven't looked at the end results yet I have been taking shoot tips and recycling them in the tissue culture. They seem to be more vigorous but I haven't looked at them under the electron microscope or used any indexing method so I cannot tell you clearly that they are free from viruses. But they should grow vigorously.

BRUCE BRIGGS: Is Dr. Harris here from Victoria, B.C.? He has been working on grapes; last year he ran about half of his grape cultivars through tissue culture and repeating the process, he felt at that time they were free of those viruses that he could identify. It would be interesting to see whether we can do this with all woody tissue, but it was done on grapes — and it is promising.

KIWIFRUIT PRODUCTION

W. H. BROKAW
Brokaw Nursery, Inc.
Saticoy, California 93004

INTRODUCTION

Of all the recently introduced subtropical crops, none has caught fire like the kiwifruit or Chinese gooseberry, *Actinidia chinensis*. Not an old warmed-over crop, or one that's been hidden in the corner, this one is a real newcomer. Since its commercial introduction by New Zealand, it has been grown commercially in the Western and Eastern United States, other American countries, Israel, Greece, Italy, France, South Africa, and Japan.

Actinidia chinensis is native to borders of the Yangtze Valley of China where it is not subject to serious frosts, but receives enough winter chill to stimulate profuse blossoms. The wild plant of these regions is a large vine that may climb to a height of 30 feet (10 m). Until recently, its popular name was *Chinese Gooseberry.*

The *Chinese Gooseberry* was brought to New Zealand about 1900, and planted as a curiosity. It remained in obscurity for years, until the New Zealanders developed certain prolific cultivars which bore abundant, highly edible fruit. The most famous of these, and the current standard, is the 'Hayward.' By the 1960's, New Zealand growers exported Chinese gooseberry fruit to the United States. About 1974 they adopted the name "kiwifruit" — presumably because of the brown hairy exterior, reminiscent of their native national bird, the kiwi.

The United States received its first pair of kiwifruit plants from New Zealand in 1935, they were planted at the USDA Plant Introduction Station at Chico, California.

The Tanimoto Brothers of Gridley first propagated kiwifruit commercially in 1964. They had heard of imported Chinese